

Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

Claim 1 (original): A method of screening for useful proteins by synthesizing a protein comprising a disulfide bond via the introduction of cysteine residues into the amino acid sequence of the protein, and analyzing the function of the protein, wherein the method comprises the steps of:

- (a) preparing one or more mRNAs encoding a protein/proteins that comprise at least two cysteine residues, and linking each of the prepared mRNAs with puromycin or a puromycin-like compound to obtain mRNA-puromycin conjugate(s);
- (b) contacting a translation system with the mRNA-puromycin conjugate(s) obtained in step (a) to synthesize the protein/proteins, and preparing mRNA-puromycin-protein conjugate(s); and
- (c) contacting one or more target substances with the mRNA-puromycin-protein conjugate(s) prepared in step (b), and determining whether the target substances interact with any one of the proteins within the mRNA-puromycin-protein conjugate(s).

Claim 2 (original): The method of claim 1, which further comprises, after step (c), the step of:

- (d) preparing DNA(s) via reverse transcription of the mRNA(s) of the mRNA-puromycin-protein conjugate(s) comprising the protein(s) that interact with the target

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substance(s), preparing mRNA(s) having a sequence that is altered by the mutagenesis of the DNA(s), and subjecting the mRNA(s) to step (a) to obtain a protein with enhanced interactive force to the target substance(s).

Claim 3 (original): A method of screening for useful proteins by synthesizing a protein comprising a disulfide bond via the introduction of cysteine residues into the amino acid sequence of the protein, and analyzing the function of the protein, wherein the method comprises the steps of:

- (a) preparing one or more mRNAs encoding a protein/proteins that comprise at least two cysteine residues, and linking each of the prepared mRNAs with puromycin or a puromycin-like compound to obtain mRNA-puromycin conjugate(s);
- (b) contacting a translation system with the mRNA-puromycin conjugate(s) obtained in step (a) to synthesize a protein/proteins, and preparing mRNA-puromycin-protein conjugate(s);
- (c) preparing DNA(s) via reverse transcription of the mRNA(s) of the mRNA-puromycin-protein conjugate(s) obtained in step (b), and preparing DNA-puromycin-protein conjugate(s); and
- (d) contacting one or more target substances with the DNA-puromycin-protein conjugate(s) prepared in step (c), and determining whether the target substances interact with any one of the proteins of the DNA-puromycin-protein conjugate(s).

Claim 4 (original): The method of claim 3, which further comprises, after step (d), the step of:

(e) preparing mRNA(s) having a sequence that is altered by the mutagenesis of the DNA(s) in the DNA-puromycin-protein conjugate(s) comprising the protein(s) that interacts/interact with the target substance(s), and subjecting the mRNA(s) to step (a) to obtain a protein having enhanced interactive force to the target substance(s).

Claim 5 (amended): The method of claim 1 or 3 [any one of claims 1 to 4], wherein, in step (a), the mRNA-puromycin conjugate(s) has/have the structure in which the 3'-terminus of the mRNA(s) is linked to puromycin or the puromycin-like compound via a spacer.

Claim 6 (amended): The method of claim 1 or 3 [any one of claims 1 to 5], wherein, in step (a), the mRNA(s) is/are prepared by transcription of DNA(s) encoding the protein(s) comprising two or more cysteine residues.

Claim 7 (amended): The method of claim 1 or 3 [any one of claims 1 to 6], wherein the translation system used in step (b) is a cell-free translation system.

Claim 8 (amended): The method of claim 1 or 3 [any one of claims 1 to 7], wherein the determination whether the target substance(s) interact with the protein(s) is

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carried out by evaluating whether the two bind to each other through the affinity column chromatography method or the affinity bead method.

Claim 9 (amended): The method of claim 1 or 3 [any one of claims 1 to 8], which further comprises the step of:

identifying the protein(s) and/or the target substance(s) that have been determined to interact to each other.

Claim 10 (amended): The method of claim 2 or 4 [any one of claims 2 and 4 to 9], wherein the mutagenesis of the DNA is achieved by the error-prone PCR.

Claim 11 (amended): The method of claim 1 or 3 [any one of claims 1 to 10], wherein a protein with enhanced interactive force to the target substance(s) is obtained by repeating each of the steps several times.

Claim 12 (amended): The method of claim 1 or 3 [any one of claims 1 to 11], wherein the mRNA(s) encodes/encode a protein/proteins comprising 8 to 500 amino acid residues.

Claim 13 (amended): The method of claim 1 or 3 [any one of claims 1 to 12], wherein the mRNA(s) encodes/encode a protein/proteins comprising 10 to 200 amino acid residues.

Claim 14 (amended): The method of claim 1 or 3 [any one of claims 1 to 13], wherein the mRNA(s) encodes/encode a protein/proteins comprising 2 to 10 cysteine residues.

Claim 15 (amended): The method of claim 1 or 3 [any one of claims 1 to 14], wherein the mRNA(s) encodes/encode a protein/proteins in which the cysteine residues adjacent to each other are separated in the amino acid sequence with an interval of 2 to 20 residues.

Claim 16 (amended): The method of claim 1 or 3 [any one of claims 1 to 15], wherein the mRNA(s) encodes/encode a protein/proteins in which the cysteine residues positioned to the most N-terminal side and the most C-terminal side are separated by 5 to 50 residues.

Claim 17 (amended): The method of claim 5 [any one of claims 5 to 16], wherein the spacer comprises, as the major backbone, polynucleotide, polyethylene, polyethylene glycol, polystyrene, peptide nucleic acid, or a combination thereof.

Claim 18 (amended): The method of claim 5 [any one of claims 5 to 17], wherein the spacer comprises a solid-phase immobilization site and wherein the mRNA-

puromycin conjugate(s) is/are linked to a solid phase via the solid-phase immobilization site.

Claim 19 (original): The method of claim 18, wherein the solid phase is selected from the group consisting of: styrene bead, glass bead, agarose bead, Sepharose bead, magnetic bead, glass base, silicon base, plastic base, metallic base, glass container, plastic container, and membrane.

Claim 20 (amended): The method of claim 18 [or 19], wherein the solid-phase immobilization site in the spacer has a cleavable site, and wherein the method comprises the step of:

folding the protein(s) on the solid phase and then cleaving the cleavable site.

Claim 21 (original): The method of claim 20, wherein the spacer is a DNA spacer and wherein the cleavable site is a restriction enzyme recognition site.

Claim 22 (amended): A synthetic protein obtainable by the method of claim 1 or 3 [any one of claims 1 to 21], which has 8 to 500 amino acid residues, comprises 2 to 10 cysteine residues for forming disulfide bonds, and has an association constant to the target substance that changes due to oxidation and reduction.

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Claim 23 (original): A synthetic protein which has 8 to 500 amino acid residues, comprises 2 to 10 cysteine residues for forming disulfide bonds, and has an association constant to the target substance that changes due to oxidation and reduction.